

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-34. (cancelled)

35. (currently amended) A modified pneumolysin polypeptide comprising one or more amino acid substitutions, wherein the modification of the in a pneumolysin polypeptide comprises substituting at least one amino acid of having a sequence of SEQ ID NO:3, and wherein said substitution is wherein single amino acid substitutions occur at a position selected from the group consisting of positions 61, 148, and 195, or wherein substitutions of multiple more than one amino acids occur at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein when said modified pneumolysin polypeptide possesses only one substitution, said substitution is selected from the group consisting of positions 61, 148, and 195, and wherein said modified pneumolysin polypeptide having at least one amino acid substitution is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity.

36. (previously presented) The polypeptide according to claim 35, wherein the hemolytic activity is less than 25% compared to wild-type pneumolysin.

37. (previously presented) The polypeptide according to claim 36, wherein the hemolytic activity is less than 1% compared to wild-type pneumolysin.

38-41. (cancelled)

42. (previously presented) The polypeptide according to claim 35, wherein the polypeptide is selected from the group consisting of pNVJ1, pNVJ20, pNVJ22, pNVJ45, pNVJ56, pNV103, pNV207, pNV111, and pNV211.

43. (previously presented) Modified pneumolysin polypeptide pNVJ1.

44. (previously presented) Modified pneumolysin polypeptide pNVJ20.

45. (previously presented) Modified pneumolysin polypeptide pNVJ22.
46. (previously presented) Modified pneumolysin polypeptide pNVJ45.
47. (previously presented) Modified pneumolysin polypeptide pNVJ56.
48. (previously presented) Modified pneumolysin polypeptide pNV103.
49. (previously presented) Modified pneumolysin polypeptide pNV207.
50. (previously presented) Modified pneumolysin polypeptide pNV111.
51. (previously presented) Modified pneumolysin polypeptide pNV211.
52. (cancelled)
53. (currently amended) A The modified pneumolysin polypeptide according to claim 35, wherein the polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, has attenuated hemolytic activity, and wherein the modification of the polypeptide is obtained by randomly mutating a nucleic acid molecule encoding a pneumolysin polypeptide, and wherein the modified pneumolysin polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide.
- 54-60. (cancelled)
61. (currently amended) The ~~conjugate~~ modified pneumolysin polypeptide conjugated to a polysaccharide according to claim ~~53~~⁶⁰, wherein the polysaccharide is derived from a bacterium selected from the group consisting of *Haemophilus influenzae* type b; meningococcus group A, B or C; group B streptococcus type Ia, Ib, II, III, V or VIII; and one or more of serotypes 1-23 of *S. pneumoniae*.
62. (currently amended) A vaccine comprising the modified pneumolysin polypeptide according to claim 35 and a pharmaceutically acceptable carrier.
63. (previously presented) The vaccine according to claim 62, wherein the polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide.
64. (previously presented) The-vaccine according to claim 63, wherein the bacterial polysaccharide is from a bacterium selected from the group consisting of

Haemophilus influenzae type b; meningococcus group A, B, or C; group A streptococcus or group B streptococcus type Ia, Ib, II, III, V, or VIII; and one or more of serotypes 1-23 of *S. pneumoniae*.

65. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein said ~~modified pneumolysin polypeptide having only~~ one amino acid substitution at position 61 is a proline or hydroxyproline substitution ~~at position 61~~.
66. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein said ~~modified pneumolysin polypeptide having only~~ one amino acid substitution at position 148 is a lysine, arginine or histidine substitution ~~at position 148~~.
67. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein said ~~modified pneumolysin polypeptide having only~~ one amino acid substitution at position 195 is a leucine, glycine, alanine, valine or isoleucine substitution ~~at position 195~~.
68. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein said ~~modified pneumolysin polypeptide having only one~~ amino acid substitution at position 243 is an arginine, valine, glutamic acid, or serine substitution ~~at position 243~~.
69. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 17, 18, 61, 66 and 101.
70. (currently amended) The modified pneumolysin polypeptide according to claim 69, wherein the substitutions consist of arginine at position 17, asparagine at position 18, proline at position 61, tyrosine at position 66, and threonine at position 101.
71. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 41, ~~[[172,]]~~ 195 and 255.

72. (currently amended) The modified pneumolysin polypeptide according to claim 71, wherein the substitutions consist of glycine at position 41, alanine at position 172, isoleucine at position 195, and glycine at position 255.
73. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 63, ~~[[127,]]~~ 128 and 148.
74. (currently amended) The modified pneumolysin polypeptide according to claim 73, wherein the substitutions consist of serine at position 63, ~~glutamic acid at position 127,~~ histidine at position 128, and lysine at position 148.
75. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 33, 46, 83, 239 and 257.
76. (currently amended) The modified pneumolysin polypeptide according to claim 75, wherein the substitutions consist of threonine at position 33, threonine at position 46, serine at position 83, arginine at position 239 and glycine at position 257.
77. (currently amended) The modified pneumolysin polypeptide according to claim 75, wherein the substitutions at positions 33, 46 and 83 are either a serine, threonine, asparagine, glutamine, tyrosine or cysteine; the substitutions at position 239 is either a lysine, arginine or histidine; and the substitution at position 257 is either a leucine, glycine, alanine, isoleucine or valine.
78. (currently amended) The modified pneumolysin polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 45, 102, 189 and 195.
79. (currently amended) The modified pneumolysin polypeptide according to claim 78, wherein the substitutions consist of alanine at position 45, glycine at position 102, arginine at position 189, and valine at position 195.
80. (currently amended) A modified pneumolysin polypeptide comprising one or more amino acid substitutions, ~~wherein the modification of the~~ in a pneumolysin

polypeptide ~~comprises substituting at least one amino acid sequence having a sequence of~~ SEQ ID NO:3, ~~and wherein said substitution is~~ wherein single amino acid substitutions occur at a position selected from the group consisting of positions 61, 148, and 195, or wherein substitutions of multiple more than one amino acid occur at positions from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, ~~and wherein when said modified pneumolysin polypeptide possesses only one substitution, said substitution is selected from the group consisting of~~ positions 61, 148, and 195, and wherein said modified pneumolysin polypeptide having at least one amino acid substitution is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity, wherein the amino acid substitution at position 17 is arginine; wherein the amino acid substitution at position 18 is asparagine; wherein the amino acid substitution at positions 33, 46, and 83 is selected from the group consisting of serine, threonine, asparagine, glutamine, tyrosine, and cysteine; wherein the amino acid substitution at position 41 is glycine; wherein the amino acid substitution at position 45 is alanine; wherein the amino acid substitution at position 61 is a proline or hydroxyproline; wherein the amino acid substitution at position 63 is serine; wherein the amino acid substitution at position 66 is tyrosine; wherein the amino acid substitution at position 101 is threonine; wherein the amino acid substitution at position 102 is glycine; ~~wherein the amino acid substitution at position 127 is glutamic acid;~~ wherein the amino acid substitution at position 148 is selected from the group consisting of lysine, arginine and histidine; ~~wherein the amino acid substitution at position 172 is alanine;~~ wherein the amino acid substitution at position 189 is arginine; wherein the amino acid substitution at position 195 is selected from the group consisting of leucine, glycine, alanine, valine and isoleucine; wherein the amino acid substitution at position 239 is selected from the group consisting of lysine, arginine, and histidine; wherein the amino acid substitution at position 243 is selected from the group consisting of arginine, valine, glutamic acid, and serine; wherein the amino acid substitution at

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position 255 is glycine; and wherein the amino acid substitution at position 257 is selected from the group consisting of leucine, glycine, alanine, isoleucine, and valine.